Comp Clin Pathol (2008) 17:203–210
DOI 10.1007/s00580-008-0724-4

ORIGINAL ARTICLE

Experimental cutaneous wound healing in rabbits: using continuous microamperage low-voltage electrical stimulation

Aboutorab Tabatabaei Naeini · Ahmad Oryan · Seifollah Dehghani · Behrooz Nikahval

Received: 14 July 2007 / Accepted: 11 February 2008 / Published online: 12 March 2008

Abstract This study was designed to determine the effects of continuous microamperage low-voltage electrical stimulation on cutaneous wound healing. Sixty mature rabbits were randomly divided into three equal groups (experimental, open control, and closed or sutured control groups). After routine surgical preparations, two 3×1 cm pieces of lumbosacral skin were excised on both sides in each animal. An incision was made over the fascia and muscle on the right side (deep wounds), and in the left side, only the skin was removed (superficial wound). Continuous direct electrical current (100 μA and 1.5 V) was applied to both wounds of the experimental group for 14 days. All rabbits were kept under observation for a period of 21 days, and their wound contraction and repair were measured daily. The rabbits then were euthanized, and biopsies were taken from the site of initial incisions. There was no significant difference in the rate of wound contraction between experimental group and open control. The yield and ultimate strength of the above mentioned specimens were lower than those of the normal skin, and the differences in biomechanical parameters between all groups were not statistically significant. There was a statistically significant decrease in the biomechanical properties of closed control lesions compared to those of the open control ($p<0.05$). Hemorrhages were evident in the upper dermis just below the epidermis, and many macrophages and lymphocytes were infiltrated at the site of injury. Electron microscopic studies showed no significant difference in the collagen fibrils diameter and distribution between different groups. There was no significant difference in the percentage dry weight of the injured skin with those of the normal skin. Results suggest that continuous microamperage low-voltage electrical stimulation, as given, did not significantly improve wound healing.

Keywords Electrical stimulation · Wound · Rabbits · Biomechanical properties · Histopathology · Electron microscopy

Abbreviations

MES	microamperage electrical stimulation
ES	electrical stimulation
HVS	high-voltage electrical stimulation

Introduction

Wound healing, the result of a complex tissue repairing process, is a continuing challenge in rehabilitation medicine. Despite some recent advances in understanding its basic principles, problems in wound healing continue to cause significant morbidity and mortality (Peacock and Cohen 1990). Living tissues possess direct-current electropotentials that appear to regulate, at least partly, the healing process (Bayat et al. 2006). After tissue injury, a current of injury is generated that is thought to trigger biological repair (Watson 1994). Exogenous electrical stimulation have been shown to enhance wound healing in both human subjects and animal models (Carley and Wainapel 1985; Brown et al. 1989; Taskan et al. 1997; Demir et al. 2004; Reger et al. 1999).

A. T. Naeini · S. Dehghani · B. Nikahval (✉)
Department of Surgery and Radiology,
School of Veterinary Medicine, Shiraz University,
P.O. Box 1731-71345, Shiraz, Iran
e-mail: nikahval@shirazu.ac.ir

A. Oryan
Department of Pathobiology, School of Veterinary Medicine,
Shiraz University,
Shiraz, Iran

Several investigators have used negative polarity low-voltage stimulation to enhance wound healing (Assimacopoulos 1968; Wu et al. 1967). Other investigators found improved healing when polarity was changed during the course of treatment (Gault and Gatens 1976). Results of the studies by Brown and Gogia (1987) using negative-polarity high-voltage stimulation (HVS) showed that the negative-polarity HVS appeared to hamper the healing process in the treated animals between 4 and 7 days postoperatively. In recent years, electrical stimulation of very low amplitude and frequency modulation has become increasingly popular treatment modality (Leffman et al. 1994). This form of stimulation has been referred to as microamperage electrical stimulation (MES). MES is defined as stimulations with a very low frequency (1 Hz or less) and low intensity or amplitude (1–1,000 μA) (Hooker 1994). Substantial evidences exists that MES applied throughout the day significantly accelerates bone healing (Friedenberg et al. 1970; Goh et al. 1988; Sharrard 1990; Oryan et al. 1996); nonetheless, the evidence that MES predictably accelerates dermal repair is less convincing (Leffman et al. 1994; Canseven and Atalay 1996; Byl et al. 1994). In view of the recent scientific understanding of the wound-healing process, one would expect a beneficial outcome from an electrotherapy that decreases edema, shorten the acute inflammatory phase, stimulates growth of fibroblasts and granulation tissue, induces epidermal cell migration, inhibits bacterial growth, decrease the ulcer size, and accelerates healing time in patients (Gentzkow 1993; Griffin et al. 1991; Carley and Wainapel 1985). Despite the theoretical basis for using MES to treat cutaneous wounds, not only no controlled, experimental studies have been conducted to demonstrate its effectiveness but also no stimulation variables used have yet been established. Recently, Bayat et al. (2006) showed that daily application of MES significantly accelerated the wound-healing process of full-thickness incision in the rabbit's skin using MES of 200 μA current intensity for 2 h/day.

This study was conducted to investigate the role of microamperage low-voltage electrical stimulation on wound healing in superficial and deep wounds when applied 24 h/day. Specifically, the study was designed to (1)assess the extent of wound closure in rabbits treated with MES and in untreated rabbits, (2)measure the tensile strength of wounds of treated and untreated rabbits, and (3) histologically examine wounds of treated and untreated rabbits.

Materials and methods

Animals

Sixty adult (10±1 months) White New Zealand rabbits of both sexes (36 males and 24 females), weighing between 1.7 and 2.3 kg, were randomly divided into three equal groups and each one housed individually in a separate standard cage with the dimensions of 45×40×50 cm. Each rabbits were randomly assigned a number that was printed on the ear with indelible ink. Animals remained in their cages, except during treatment setup, where standard rabbit diet and water were available ad libitum. Temperature (25°C) and the ratio of daylight hours to non-daylight hours (12 h light/12 h dark) were kept constant.

Rabbits were divided into one experimental group and two control groups comprising of the experimental group (n=20): first control group, which was open wound control group (n=20), and second control group, which was closed (sutured) control group (n=20). Each group was divided into four equal subgroups of five rabbits for different studies (Table 1). All animals were followed for 21 days of the experiment. Finally, biopsies were taken from the site of

Table 1 Division of animals per groups and subgroups, number of samples of each subgroup, and the aim of sampling for each subgroup

Groups	Subgroups	Superficial wound samples	Deep wound samples	Purpose of sampling	Total samples per each subgroups[a]
Experimental group (n=20)	n=5	5	5	Histopathology	10
	n=5	5	5	Biomechanics	10
	n=5	5	5	Electron microscopy	10
	n=5	5	5	Percentage of dry weight	10
Open control group (n=20)	n=5	5	5	Histopathology	10
	n=5	5	5	Biomechanics	10
	n=5	5	5	Electron microscopy	10
	n=5	5	5	Percentage of dry weight	10
Closed control group (n=20)	n=5	5	5	Histopathology	10
	n=5	5	5	Biomechanics	10
	n=5	5	5	Electron Microscopy	10
	n=5	5	5	Percentage of dry weight	10

[a] Totally 30 histopathological samples, 30 biomechanical samples, 30 EM samples, and 30 samples for percentage dry weight measurements were used.

initial excision from the animals of all subgroups for biomechanical, histopathological, and ultrastructural studies. The percentage dry weight was also determined.

This study was approved by the ethical committee of Veterinary School of Shiraz University, and the principles of laboratory animal care (NIH publication NO. 86–23, revised 1985) were followed.

Surgical procedure

The rabbits of both experimental and control groups were anesthetized by injection of 20 mg/kg ketamine hydrochloride intravenously in the marginal ear vein. Before making incisions, the dorsal aspect of the lumbar area was shaved and washed with scrub solution of povidone iodine. Under sterile conditions, skin incisions were made in a rectangular shape 3 to 1 cm, perpendicular to the vertebral column in both sides, and the cranial border 1 cm caudal to the last rib; then, the skin was excised. In the left side, only the skin was removed (a superficial wound), but in the right side, the underlying tissues such as subcutaneous fascia, panniculus carnosus, and lumbar muscles were also excised (deep wound). The same procedures were performed for experimental and control groups. The duration of anesthesia was about 15 min for each rabbit.

Treatment regimes

In the open wound control group, after making the incisions on both sides, no treatment was applied on the incisions, the incisions were remained intact, and daily repair and wound contraction were measured. In the closed wound control group, everything was similar to those of the untreated open wound controls except that the skin incisions were sutured using no. 0. with silk material in simple interrupted pattern.

In the experimental group, after making superficial and deep wounds, direct current (DC) electrical stimulation with low intensity was applied for each incision separately. The electrical stimulation device consisted of two alkaline batteries (1.5 V), as current sources, variable resistors (5 kΩ), and connecting wires. Two negative electrodes were applied to both sides of the long axis in the margin of the ulcer, using Michel clips, but the positive electrode was applied by inserting a no. 20 stainless steel wire in the middle of the two negative electrodes and in the middle of the ulcer. After attaching the electrodes, a continuous electrical stimulation was applied continuously for a duration of 14 days. The intensity of DC was set to 100± 5μA with digital micrometer. The observation of the electrostimulation device attachments was performed three times a day, and the attachments were removed at the end of the day 14 postoperation.

Sampling

At the end of day 21 postoperation, all rabbits were euthanized by IV injection of 40 mg/kg thiopental sodium (Nesdonal)® via marginal ear vein and sampling was done. At this time, samples from one subgroup of each groups were collected for histology, and each of the second, third, and fourth subgroups of each groups were designed for electron microscopic studies, percentage dry weight analysis, and biomechanical studies, respectively. For each study, 30 samples were collected and studied [e.g., for histological studies, ten samples of experimental group (five of them from deep wounds and five from superficial wounds) and ten samples from open control (five deep and five superficial) and ten samples from closed control were used].

Sampling for biomechanical studies

After shaving, the skin containing the incision area was excised in a rectangular shape (12×2 cm). Another similar skin sample from the intact skin of the lumbar region far from the site of initial excision of the same animal was excised as normal skin control. The samples were kept frozen (−18°C), promptly after sampling for a maximum of 5 days before being tested (Butler et al. 1978; Woo et al. 1980; Oryan and Zaker 1988). A Universal Instron testing machine was used to determine the biomechanical characterization (TT-CM-L, England). After thawing, the skin pieces were mounted on a frame and made certain the skin was not stretched in any direction, and then both sides of the skin flap were clamped into a pair of grips, with the wound in the middle of the skin piece. The skin flap was stretched using a constant speed of 10 mm/min and a chart speed of 5 cm/min, and the yield strength and ultimate strength were calculated based on the load–deformation curve.

Histopathological studies

Skin samples were taken from both the wound and adjoining normal skin and fixed in 10% neutral-buffered formalin. After fixation, the tissues were embedded in paraffin, and 5 μm thickness sections were stained using hematoxylin and eosin (H&E), Alcian blue/periodic acid-Schiff (PAS) to visualize glycosaminoglycans, Masson's Green Trichrome, and Van Gieson and Verhoeffs for collagen and elastic fibers. Ten zones were examined from the sample morphometrically through a calibrated ocular on a Nikon light microscope (Nikon, Tokyo, Japan) at a magnification of ×400. Histological examinations were performed in a double-blind fashion. The criteria that were studied in histopathological sections consisted of hemorrhage, fibrin deposition, polymorphonuclear cell and mononuclear cell infiltration, reepithelialization, cornification

Table 2 Mean (±SD) of wound-closure time

Group	*n*	Deep wound closure time[a] (right; day)	Superficial wound closure time[a] (left; days)
Open wound control group	20	17.50±3.02	15.50±3.33
Experimental group	20	17.25±3.15	14.87±2.47

n Number of animals from which wound-closure values obtained.
[a] Wound-closure time are not significant

of the epithelium, fibroblast content, glycosaminoglycan secretions, collagen content, revascularizations, necrosis, presence of fibrocytes, and maturation and organization of collagen and elastic fibers.

Electron microscopic studies

Skin samples of 1×1×1 mm dimensions were fixed using 2% cold glutaraldehyde and 2% paraformaldehyde in 0.125 M sodium cacodylate buffer at pH 7.4 (Karnovsky 1965). The tissues were then post-fixed in 1% osmium tetroxide in 0.1 M sodium phosphate, pH 7.4 for 1 h, dehydrated in graded ethanol, and thereafter embedded in Spurr resin. Semithin sections of 1 μm in thickness were stained with 0.25% toluidine blue for 30 s and observed with a Nikon Labophot AFX-II light microscope. Representative areas were selected, then ultra thin sections of 70–80 nm in thicknesses were stained with uranyl acetate and counterstained with lead citrate. After the staining process, the samples were observed with an electron microscope (Phillips, M300, The Netherlands).

Statistical analysis

One-way analysis of variance and Duncan's multiple range test were used to evaluate the differences of biomechanical parameters and dry weight content between experimental and control groups (in each comparison, deep and superficial wound of experimental group compared to the similar wounds of control groups, otherwise mentioned). Student's *t* test was used to evaluate the significant differences of biomechanical parameters and dry weight contents between the normal skin sample and deep wounds, between superficial wounds and normal skin, and also between superficial and deep wounds. Differences were considered significant when $p<0.05$, using computer software SPSS version 11.5 for windows (SPSS, Chicago, IL, USA).

Results

Wound closure

The time that the edges of the wound reached each other considered as wound-closure time, which was measured daily and described as mean (±SD). There was no significant difference in wound-closure time between open control and experimental groups (Table 2).

Tensile strength

As is shown in Figs. 1 and 2, there was a significant decrease in yield and ultimate strength in the superficial and deep wounds of all groups as compared to normal skins. The differences in deep wounds between all groups were not significant in both yield and ultimate strengths. In superficial wounds, the differences were statistically higher in open control as compared to closed control ($p<0.05$) but not significant between control and experimental groups.

Histopathological findings

In both untreated deep and superficial open wounds, the re-epithelialization and cornification were optimal and both of them were infiltrated mildly with lymphocytes, plasma cells, macrophages, and neutrophils; however, cell infiltration were greater in control deep wounds compared to those of the untreated superficial ones (Fig. 3). An increase in the number of the fibroblasts was evident in both deep and superficial wounds of open control group, and the fibroblasts showed the characteristics of the mature cells and some of them were very similar to fibrocytes. Alcian blue/PAS staining showed an increase in glycosaminoglycan and proteoglycan concentrations of the ground substance of both deep and superficial control wounds, but the newly formed collagen fibers were still unorganized and haphazardly distributed. Angiogenesis

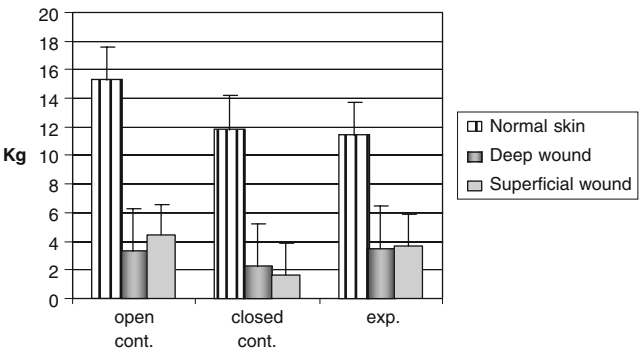

Fig. 1 Yield strength. The difference was not significant between control and experimental groups ($p>0.05$). The differences between deep and superficial wounds in all groups were not significant

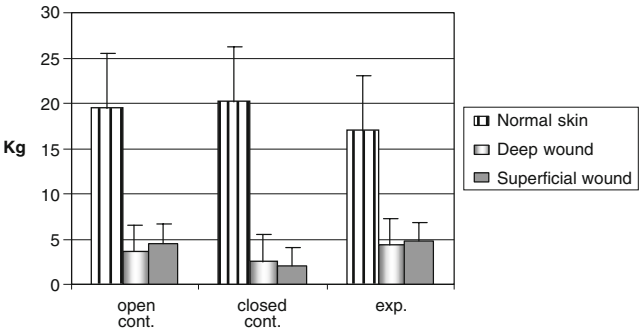

Fig. 2 Ultimate strength. The difference was not significant between control and experimental groups ($p>0.05$)

was evident in both deep and superficial untreated wounds, and the number of blood vessels was greater compared to those of normal skins. No elastic fiber was seen after staining open superficial and deep control wounds with Van Geison and Verhoeffs. However, more polymorphonuclear and mononuclear inflammatory cells were infiltrated in closed deep and superficial wounds compared to those of the open ones. The suture areas of these wounds were still not complete, and the newly formed connective tissue and blood vessels were more unorganized compared to those of untreated open wounds (Fig. 4). However, re-epithelialization and cornification were complete in both of these wounds too. The treated animals showed fewer polymorphonuclear and mononuclear cell infiltration and a better tissue alignment compared to those of untreated animals. Re-epithelialization and cornification were optimal, and the concentration of glycosaminoglycans and proteoglycans of the matrix were less prominent compared to those of untreated groups (Fig. 5). Except the above criteria, the rest factors were similar in both treated and untreated animals.

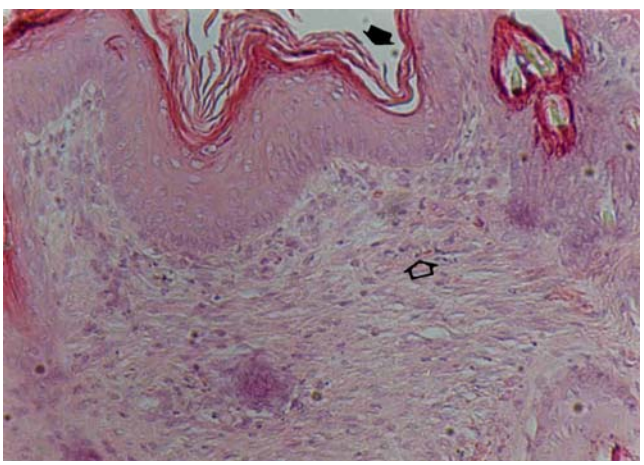

Fig. 3 Deep wound in open control group. Re-epithelialization and cornification (*black arrow*). Newly formed connective tissue and blood vessels in dermis mildly infiltrated with leukocytes (*white arrow*) (H&E staining, ×148)

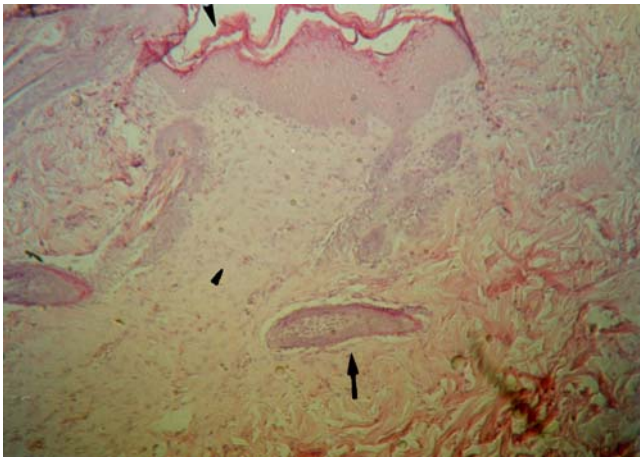

Fig. 4 Deep wound in closed control group. Re-epithelialization and cornification (*big arrowhead*). Newly formed connective tissue and blood vessels in dermis are more unorganized compared to open control ones (*small arrowhead*). Suture area is still present in the wound region (*arrow*) (H&E staining, ×92.5)

Ultrastructural findings

Collagen fibrils in electron micrographs were seen as long and thin fibrils that were separate from each other. It was not possible to measure the length of the collagen fibrils in the sections, but the diameters of the fibrils were measured. Normal rabbit skin exhibited a bimodal distribution of collagen fibril diameters (Fig. 6). The larger collagen fibrils were the major population, which had a maximum diameter of 160 nm and the diameter of the smaller fibrils were about 32–35 nm. Mean (±SD) of diameter of collagen fibrils are presented in Table 3.

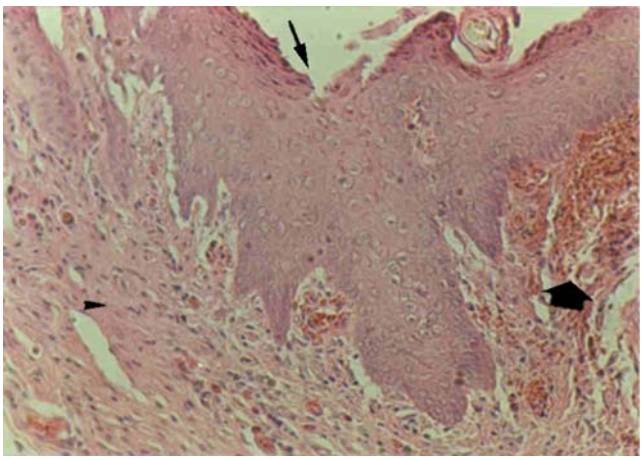

Fig. 5 Deep wound in experimental group. Re-epithelialization and new formed keratin layer on the surface of epidermis (*thin arrow*). The fibrous connective tissue of the dermis is still unorganized and is infiltrated with mononuclear cell infiltration (*arrowhead*). Sub epithelial hemorrhage is present (*thick arrow*) (H&E staining, 233)

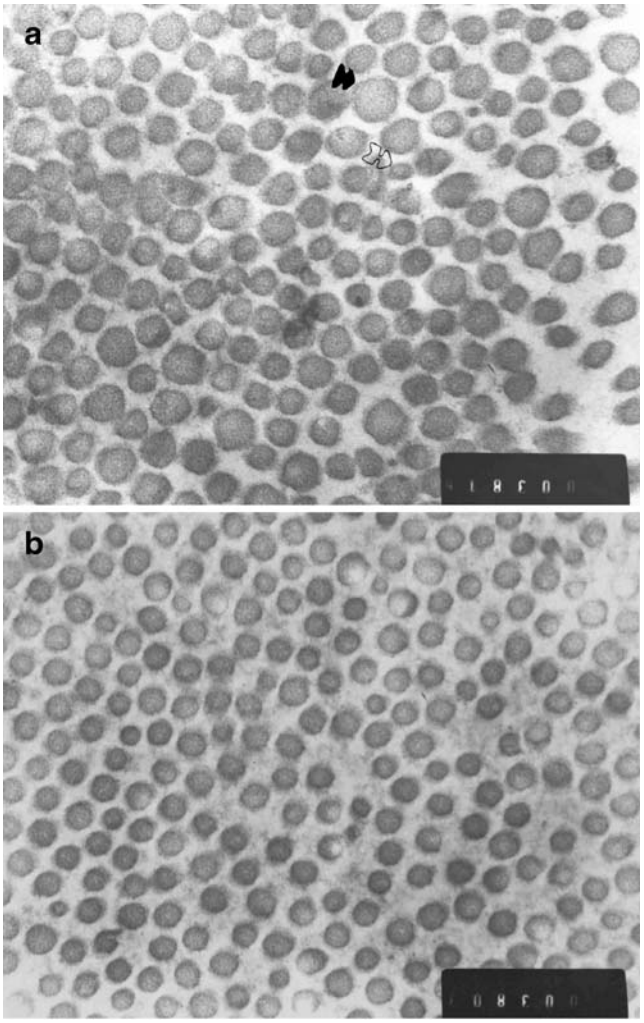

Fig. 6 **a** Ultra micrograph of normal rabbit skin of rabbits. The larger collagen fibrils (*black arrow*), and the smaller collagen fibrils (*white arrow*). **b** Deep wound of experimental group is converting to bimodal distribution (×56,100)

Discussion

This study was designed to determine whether continuous microamperage low-voltage electrical stimulation is an effective means of promoting wound healing. Yarkony (1994), having reviewed medical management of pressure ulcers, reported that although several studies have been published on the effects of electrical stimulation on wound healing process, many of them either had a poor sample size or were poorly controlled. The failure of these techniques for clinical use is important because a therapist may require applying electrical stimulation. In the present study, wound-closure data for experimental group as compared to open control group did not support the use of microamperage low-voltage electrical estimulation, as given to enhance wound healing. Results of the study by Brown and Gogia (1987) using negative-polarity HVS on full thickness incisions on the back of the rabbits showed that there was no significant difference in the percentage of wound closure between experimental and control groups in days 4 and 7 post-incision, and the rabbits in control group had a nonsignificant higher percentage of wound closure than the experimental group. Brown et al. (1988) showed that using positive polarity high-voltage stimulation on full thickness incisions on the back of the rabbits causes wound closure for experimental group (50%) to be significantly lower than those of the control group (78%) at 4 days post-incision. After 7 days, however, the rabbits of both experimental and control groups had similar wound-closure values (80% and 82%, respectively). Assimacopoulos (1968) showed 25% increase in wound healing in full thickness incisions using negative-polarity direct current. Lower yield and ultimate strength of superficial and deep wounds in experimental group as compared to normal skin indicate that after electrical stimulation, the biomechanical properties did not reach the normal values, and also there was no significant differences between experimental and control groups. Brown et al. (1988) did not find significant difference between rabbits treated with negative-polarity direct current and control group. They also indicated that application of electrical stimulation did not enhance wound healing during the first 4 days after incision and delayed wound healing process 7 days post injury. They stated that the lack of effectiveness of electrical stimulation may be related to numerous factors, including polarity used, physical, ionic and electromagnetic influences, stimulation characteristics, method used, and the small sample size. Findings of the present experiment are in accordance with those of Brown and Gogia (1987) and Alvarez et al. (1983). Alvarez et al. (1983) stated that the tensile strength value measuring was a physical measurement and was associated with collagen cross-links and not with collagen synthesis. Electricity may alter collagen synthesis but does not have an effect on maturation and organization of collagen fibrils. These findings are in accordance with our biomechanical results.

Histopathological results showed proper re-epithelialization and cornification of the epithelium, fewer inflammatory cells

Table 3 Mean (±SD) of diameter of collagen fibrils measured in nm in ultramicrographs

Group	*n*	Deep wound (nm)[a]	Superficial wound (nm)[a]
Open wound control group	5	108.98±24.12	107.90±22.79
Closed wound control group	5	102.14±33.44	101.42±29.02
Experimental group	5	104.12±17.99	105.20±26.89

Mean diameter of collagen fibrils in normal skin: 127.70±29.15.
n Number of animals per group
[a] There are no significant difference between control and experiment group.

infiltration, and better collagen fibrils alignment in the dermis and subcutis of the experimental group. Hemorrhage in the experimental group at the end of the day 21 may be due to several reasons, such as direct insertion of positive electrode in the incision site, effect of electric current on the scar tissue, and tissue irritation by stainless steel products. Nessler and Mass (1987) reported necrosis and hemorrhage in scar tissue in ligaments stimulated by electrical current. Brown and Gogia (1987) and Brown et al. (1988) did not find a significant difference in collagen content and cell accumulations between the group treated by electric current and the control group. Brown et al. (1988) showed that electrical current increased the epithelial thickness of skin wounds as compared to control group. The electron microscopic results revealed no significant difference in collagen fibrils diameter in superficial and deep wounds as compared to control group. In electrically stimulated animals similar to open control group, the collagen fibrils diameter did not reach their maximum diameters and were smaller than those of normal skin. Bourgiugnon and Bourgiugnon (1987) studied protein and DNA synthesis by fibroblasts in cell culture with scanning electron microscopy and showed alternating current (50 and 75 V) had positive effects on protein and DNA synthesis, but voltages higher than 300 V reduced protein and DNA synthesis.

There was no significant difference between the percentage dry weight content of the skin lesions of experimental and control groups; however, both treated and untreated lesions had higher water content than those of normal skin. Increase in the water content may be due to inflammatory reaction, edema formation, increase in collagen Type III formation, and decrease in total collagen contents of the treated and untreated wounds.

In the papers that reported positive effects of ES, most of them were on chronic ulcers (Gault and Gatens 1976; Griffin et al. 1991; Brown et al. 1988). The present study described the application of ES on acute wounds; therefore, it is beneficial to evaluate the effect of ES as such a modality on chronic wounds. Having used continuous microamperage low-voltage electrical stimulation in rabbits in light of the aforementioned statements to accelerate wound healing, we concluded that continuous application of electrical stimulation as given on surgically induced incisional wounds do not significantly alter wound healing process. However, further investigations are required to address the stimulation effect in other modalities in animals.

Acknowledgments The authors are grateful for financially support of this research by a grant from Veterinary School and Shiraz University Research Council. We are also indebted to B. Shourangiz from the Pathobiology Department of The Veterinary School, Y. Adeli and As. M. Zakeri from the Biology Department of the Basic Science School, and M. Mahzoon from the Material Department of Engineering School for their technical assistance.

References

Alvarez OM, Mertz PM, Smerbeck RV et al (1983) The healing of superficial skin wounds is stimulated by external electrical current. J Invest Dermatol 81:144–148

Assimacopoulos D (1968) Wound healing promotion by the use of negative electric current. Am Surg 34:423–431

Bayat M, Asgari- Moghadam Z, Maroufi M et al (2006) Experimental wound healing using microamperage stimulation in rabbits. J Rehabil Res Dev 43:219–226

Bourgiugnon GJ, Bourgiugnon LY (1987) Electric stimulation of protein and DNA synthesis in human fibroblasts. FASEB J 1:398–402

Brown M, Gogia PP (1987) Effects of high voltage stimulation on Cutaneous wound healingin rabbits. Phys Ther 67:662–667

Brown M, McDonnel MK, Menton DN (1988) Electrical stimulation effect on cutaneous wound healing in rabbits. Phys Ther 68:655–660

Brown M, McDonnel MK, Menton DN (1989) Polarity effects on wound healing using electric stimulation in rabbits. Arch Phys Med Rehabil 70:624–627

Butler DL, Grood ES, Noyes FR (1978) Biomechanics of ligaments and tendons. Exerc Sport Sc Rev 6:125–181

Byl NN, Mckenzie AL, West JM et al (1994) Pulsed microamperage stimulation: a controlled study of healing of surgically induced wounds in Yucatan pigs. Phys Ther 74:201–219 Erratum in: Phys Ther 1994; 74: 595)

Canseven AG, Atalay NS (1996) It is possible to trigger collagen synthesis by electric current in skin wounds. Indian J Biochem Biophys 33:223–227

Carley PJ, Wainapel SF (1985) Electrotherapy for acceleration of wound healing: low intensity direct current. Arch Phys Med Rehabil 66:443–446

Demir H, Balay H, Kirnap M (2004) A comparative study of the effects of electrical stimulation and laser treatment on experimental wound healing in rats. J Rehabil Res Dev 41:147–154

Friedenberg ZB, Andrews T, Smolenski BI et al (1970) Bone reaction to varying amounts of direct current. Surg Gynecol Obstet 131:894–899

Gault WR, Gatens PF (1976) Use of low intensity direct current in management of ischemic skin ulcers. Phys Ther 56:265–269

Gentzkow GD (1993) Electrical stimulation to heal dermal wounds. J Dermatol Surg Oncol 19:753–758

Goh JC, Bose K, Kang YK et al (1988) Effects of electrical stimulation on the biomechanical properties of facture healing in rabbits. Clin Orthop Relat Res 233:268–273

Griffin JW, Tooms RE, Mendius RA et al (1991) Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. Phys Ther 71:433–442

Hooker DN (1994) Electrical stimulation currents. In: Prentice WE (ed) Therapeutic modalities in sport medicine. William, Brown Co., St. Louis, pp 51–88

Karnovsky MJ (1965) A formaldehyde-glutaralehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol 27:137

Leffman DJ, Arnall DA, Holmgren PR et al (1994) Effect of microamperage stimulation on the rate of wound healing in rats: a historical study. Phys Ther 74:195–200

Nessler JP, Mass DP (1987) Direct-current electrical stimulation of tendon healing in vitro. Clin Orthop Relat Res 217:303–312

Oryan A, Zaker SR (1988) Effects of topical application of honey on cutaneous wound healing in rabbits. J Vet Med A 45:181–188

Oryan A, Meimandi Parizi AH, Dehghani S (1996) Biomechanical and ultrastructural studies on effects of electrical stimulation on experimental fracture healing in rabbit. Indian J Vet Surg 17:98–105

Peacock EE, Cohen IK (1990) Wound healing. In: McCarthy JG, May JW, Littler JW (eds) Plastic surgery. Saunders, Philadelphia, pp 161–185

Reger SI, Hyodo A, Negami S et al (1999) Experimental wound healing with electrical stimulation. Artif Organs 23:460–462

Sharrard WJ (1990) A double-blind trial of pulsed electromagnetic fields for delayed union of tibial fractures. J Bone Joint Surg Br 72:347–355

Taskan I, Ozyazgan I, Tercan M et al (1997) A comparative study of the effects of ultrasound and electric stimulation on wound healing in rats. Plastic Recnstr Surg 100:966–972

Watson T (1994) Electrical stimulation for wound healing. In: Bazin S, Kitchen S (eds) Clayton's electrotherapy. Saunders, Philadelphia, pp 223–397

Woo SLY, Ritter MA, Amiel D et al (1980) The biomechanical and biochemical properties of swine tendon- long term effects of exercise on the digital extensors. Conn Tissue Res 7:177–183

Wu KT, Go N, Dennis C et al (1967) Effects of electric currents and interfacial potentials on wound healing. J Surg Res 7:122–128

Yarkony GM (1994) Pressure ulcers: a review. Arch Phys Med Rehabil 75:908–917